

STUDIES ON CEREBRO-SPINAL FLUID. NO. III.*

THE PATHWAYS OF ESCAPE FROM THE SUBARACHNOID SPACES WITH PARTICULAR REFERENCE TO THE ARACHNOID VILLI.

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In the preceding communication of this study the more recent literature concerning the drainage of the cerebro-spinal fluid from the subarachnoid space was presented, together with a more or less critical analysis of the methods

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of investigation. It was pointed out that the later observations on this subject incline toward the belief that the fluid is, for the most part, returned to the general circulation by way of the cerebral sinuses and to a smaller degree by the lymph channels. The detailed proof of this pathway was not presented except in the reports of Key and Retzius. Much of the work has been done with methods which possess such disadvantages that any conclusions drawn from the findings are apt to be erroneous. On theoretical grounds, the advantages of introducing true solutions, capable of subsequent precipitation in situ and of microscopic identification, were discussed, and a method of study based on the well-known Prussian blue reaction was given in detail. The non-toxicity of the ferrocyanide solution on intraspinal injection, the non-absorption of the fluid as a diffuse tissue stain, and the ease of subsequent precipitation and microscopic study, — these are the factors which led to the conclusion that the method possessed distinct advantages in the study of the absorption of cerebro-spinal fluid.

In addition to the comment upon the various methods of investigation, a protocol giving in part the gross and microscopic findings after a low pressure intraspinal injection of the ferrocyanide solution was included. Most important of the observations recorded was the undoubted passage of the fluid directly into the great sagittal sinus by way of villi of arachnoidal origin and morphology. With this fundamental finding before us it will now be possible to report the exact pathways of fluid elimination from the meningeal spaces.

This communication concerns a detailed account of the results of these observations on the escape of cerebro-spinal fluid from the subarachnoid space in the cranial and spinal portions. A comparison of the findings after employing various approaches, with varying pressures and with different methods, will be given; for different methods yield different results in the hands of different observers and it is only by comparing the findings with these different methods in the hands of one worker that accurate deductions may be made.

I. THE CHIEF PATHWAY OF ESCAPE INTO THE CEREBRAL SINUSES.

(*a.*) Histological evidence of valvular mechanism not found. — As much of the evidence presented by previous workers in this field indicated that the dural sinuses were concerned with the cerebro-spinal drainage, and that possibly a valvular mechanism existed, this study began with an attempt to find histological evidence of such a valve-like arrangement about the superior longitudinal sinus. Serial coronal sections of this great venous channel were examined; the results in this regard were uniformly negative. The careful study of these serial sections failed to afford any histological evidence of a valvular mechanism concerned in the drainage of cerebro-spinal fluid; as for the physiological evidence, the findings are somewhat different and will be given an appropriate place in this communication.

(*b.*) Injections of suspended granules into cerebro-spinal spaces. — As we became early convinced that by histological methods alone but little could be ascertained regarding the pathway of physiological drainage, our observations were next concerned with the injection of granular material into the spinal subarachnoid space. It was believed that reliable evidence regarding the physiological method of absorption could be obtained only when the experimental conditions approximated the normal. Hence, in animals anesthetized with chloretone or with ether given by intratracheal insufflation the lower thoracic cord was exposed by a laminectomy. After transection of the spinal cord a cannula was tied in the subarachnoid space and injections were made at pressures slightly above the normal cerebro-spinal tension (*i.e.*, 130 to 180 mm. H₂O). These were continued for periods of four to six hours; constant pressure systems were used so that the fluid absorbed did not affect the pressure. The animals were maintained under complete anesthesia throughout the period of observation. Suspensions of lampblack, finely ground and filtered, in saline solution

were first employed; these were replaced in subsequent experiments by diluted india ink (in which the granules of carbon are even more finely divided). At the end of the period of injection the spinal cord and brain were removed in one piece, keeping intact the meninges, and the tissue block was then hardened in ten per cent formol.

By this method of long continued injection under low pressures both suspensions yielded approximately identical results. Subsequently, on macroscopic examination, the black granules could be followed in the spinal subarachnoid space upward to the cisternæ about the cerebellum and to all of the basilar pockets of fluid. But over the cerebral convexities no granules could be seen with the naked eye. The amount of this granular material was not great as one passed from the region of injection; on microscopic examination the reason for this became apparent. The granules were caught and apparently held firmly in the meshes of the arachnoid network. The granules were not actively phagocytosed in the time of injection, but many of them were contained in the arachnoid network. This retention of the granules is in accord with the observations of Quincke, and of Sicard and Cestan, but in their experiments the longer duration of the observations had afforded opportunity for phagocytosis to occur. Goldmann comments upon the probability of the "pyrrol-zellen" playing the essential part in this process of phagocytosis within the meninges.

It was with considerable interest that the walls of the superior longitudinal sinus were studied in this series of injections. But the only positive finding was that of an occasional granule of carbon in the midst of cellular cords in the dense strands of dural connective tissue, and throughout a whole series only one or two small granules were found within the cytoplasm of an endothelial cell in the lining wall of the sinus. The lungs of the animals were studied, but without result; the frequent anthracosis could too readily be confused with the injected granules. Sections of the optic nerves revealed granules in the subarachnoid prolongation, and others of the cranial nerves showed similar

injections for short distances away from the cerebro-spinal axis. The cervical lymph nodes in this series contained no carbon granules.

Realizing the great disadvantage of a granular mass because of its tendency to be caught in tissue meshes and to be phagocyted, yet admitting its value because of easy microscopic identification, we next made use of a precipitated carmine suspension. Maintaining the insolubility of the carmine by keeping the solution slightly acid in reaction, injections were made in approximately the same method as given above. The results were practically identical, except that in the lung on frozen section a few characteristic carmine granules were found, but in such small number that it was apparently only a chance discovery, and perhaps one granule in a thousand had passed into the general circulation. The identification of these solitary granules was sufficient to suggest that, either by phagocytosis or by other means of passage through a cellular meshwork, a granular substance could occasionally be passed out of the cerebro-spinal spaces.

(*c.*) The results of the injection of true solutions.—The heaping up and the retention of the granular substances in the meshes of the arachnoidea made it clear that such introductions could not advance the problem. For when one examines the evidence showing the formation and absorption of cerebro-spinal fluid (the circulation of Cathelin⁷), the inevitability of a free passageway for fluids becomes granted. Hence, our attention was turned to the possibility of injecting a true solution with subsequent precipitation. Many solutions (carmine, silver nitrate) were tried, but all proved unsatisfactory because of their toxicity or because of their action as diffuse tissue stains. Not until trials were made with potassium ferrocyanide and iron ammonium citrate were satisfactory results obtained.

The technic of the low pressure injections and of our use of the solution of potassium ferrocyanide and iron ammonium citrate has been reported in the foregoing

communication in this study. It is not proposed to record here any protocols but to discuss in detail our findings in the various phases of the problem. For the most part, our injections have been made with pressures just far enough above the normal tension to secure replacement of the cerebro-spinal fluid with the foreign solution. It was soon found that if an injection be continued under these pressures for three hours, a complete injection of the spaces could be secured and most of our experiments were planned on this basis. Variations from this typical method of study will be commented upon whenever they afford insight into any particular feature of the problem.

The characteristic gross findings in a typical subarachnoid injection are the filling of the diploetic vessels with a Prussian blue deposit, the pale tinting of the dura, the marked outlining of the arachnoid channels, the perineural injection of the cranial nerves, the coloring of the cervical nodes and lymph channels, the bluish deposits in the nasal mucosa and in the lining walls of the cranial air cells. They will be discussed in appropriate paragraphs of this communication.

(*d.*) The subdural space. — Probably the first feature to attract attention on microscopic examination after a ferrocyanide injection in the spinal subarachnoid space is the absence of the precipitation in the subdural space. In the specimens resulting from the injection under pressures but slightly increased over the normal, the freedom of this space from granular content is very marked (Figs. 1, 2, 3, etc.).

The arachnoid spaces over the convexity of the brain may be very completely filled with precipitated granules, but these do not appear subdurally. This is in complete accord with the observations of Key and Retzius,²⁴ based upon the injection of gelatine masses into the spinal subarachnoid space. Typically, the Prussian blue deposit, in greatest part, adheres closely to the mesothelial covering of the different strands of arachnoid tissue; some, however, clings to the pial cells, and some even appears free in the fluid spaces. This

tendency of the granules to adhere to the cells of the arachnoidea must be explained on the basis of the physical conditions existing at the time of precipitation, for the granules merely cling to the surface of the cells and are not within the cell-body itself. This phenomenon is shown in a low power drawing (Fig. 1) by the presence of a clear zone of arachnoid mesothelium covering the marked subarachnoid collections. Hence, it may be considered that these arachnoid mesothelial cells form an efficient protection against any fluid absorption by the underlying tissues; for physiological purposes they may be assumed to be impermeable to the ferrocyanide solution. (In many of the preparations in which high pressures (50 mm. Hg.) were employed, the mesothelial arachnoid cells show within their cytoplasm collections of minute Prussian blue granules. None of these granules are, however, passed into the subdural space, so that their presence in the arachnoidal cells apparently is due to a specific physical (possible chemical) property rather than a phenomenon due to fluid passage through the membrane.) This undoubtedly explains the absence of fluid in the subdural space under normal conditions; with a covering about the subarachnoid spaces, impermeable to fluids, it seems more than likely that whatever fluid is present in the subdural space is the product of the activity of the cells lining this serous cavity, and is not cerebro-spinal fluid which has passed from the subarachnoid space through the arachnoid membrane into the subdural space. On the other hand, it seems probable that any fluid which may accumulate in the subdural space is absorbed chiefly by way of the subarachnoid pathway; this phase of the problem will be considered later. Quincke's⁴⁰ observations of the passage of cinnabar granules from subdural into subarachnoid spaces accord with this view, for never was he able to demonstrate the reverse direction of passage.

(*e.*) The cortical subarachnoid space.—The subarachnoid space over the cerebral hemispheres extends deeply into the cerebral sulci, while the arachnoid membrane bridges

these furrows from gyrus to gyrus. Over the convolutions the subarachnoid space is only of capillary thickness until it dips again into a fissure. It is not strange, then, that most of the granular collections, after a typical low-pressure injection, appear in the spaces about the sulci in contrast to the delicate sheet of granules which covers the surface of the gyri. Over the entrances of the sulci, where the arachnoid membrane is widely separated from pia mater, the inner and outer mesothelial linings of the membrane become very evident. The inner mesothelium apparently serves as the cellular lining of the cerebro-spinal fluid spaces, whereas the outer covering prevents adherence to the dura and is related in some way to the capillary fluid layer in the subdural space.

(*f.*) Different distributions of the injection masses.—Injections of the ferrocyanide solutions at pressures but slightly above normal (120–180 mm. H₂O) resulted in different granular distributions dependent upon time and pressure factors. If such an injection be continued only for an hour or less, the precipitated granules are found chiefly in the basilar cisternæ in the cranial chamber and only in slightest traces over the cerebral hemispheres. These specimens show the Prussian blue granules in the arachnoid villi in the cavernous sinus in large numbers and only in slight amounts in the villi of the superior longitudinal sinus. This finding is in keeping with the observations of Goldmann,¹⁶ after intraspinal injection of trypan-blue. He pictures the basilar distribution of the dyestuff with but little spread over the hemispheres. Similarly, we have found that if we withdraw one or two cubic centimeters of lumbar cerebro-spinal fluid and replace this immediately with an equal quantity of a ferrocyanide solution, the same basilar distribution of the Prussian blue will be found after an hour in the cranial cavity with extensive passage of the fluid into the cavernous sinus through the villi. However, if such a replacement be carried out in the cerebellar cisternæ, the whole subarachnoid space over the hemispheres becomes filled with the ferrocyanide solution and extensive drainage into the superior

longitudinal sinus takes place. Goldmann found a similar rapid spread of his dyestuff when injected in the cranial subarachnoid spaces, both hemispheres being almost instantly suffused.

We have been able to secure a complete and widespread distribution of the injection masses, especially those which are true solutions, by continuing the injection under pressures just above normal for several hours. The pressures are such that they secure merely a displacement of the normal fluid throughout the cerebro-spinal system. Similarly, a complete and rapid subarachnoid injection, with passage of the solution into the cerebral sinuses through arachnoid villi, may be secured by injecting ten cubic centimeters into the subarachnoid space under a pressure of fifty millimeters of mercury.

Results such as these with different pressure factors, with different points of injection, and with different periods of injection, are of great importance in the final solutions of the problems of cerebro-spinal drainage. Apparently the fluid from the spinal canal first seeks the basilar systems, while that introduced in the cerebellar cisternæ or above the tentorium flows toward the superior longitudinal sinus. Long continued injections in the spinal regions at pressures slightly above normal, or at higher pressures for shorter periods, suffice to cause a further spreading of the injection fluids from the primary basilar receptacles over the hemispheres. Whatever the distribution of the injection fluid may be, it is possible in every case to demonstrate its absorption through arachnoid villi into the cerebral sinuses.

(*g.*) Escape of fluid into cerebral veins (?). — The blood vessels which traverse the subarachnoid space are covered by arachnoidal mesothelium on the outer surface of the adventitia. As far as can be made out from study of the preparations injected at normal pressures no granules appear in the lumen of the vessels, nor are any seen traversing the mesothelium and vessel wall. This indicates that normally the cerebral (in contradistinction to the pachymeningeal)

veins play no part in the absorption of cerebro-spinal fluid. Even should the Prussian blue precipitate be made out in the cerebral veins, the possibility of a retrograde injection would have to be considered. This possibility has been eliminated in these observations by making the injections during the life of the animal, and it may well be assumed that the blood flow through the veins and sinuses is sufficient to prevent retrograde injections in these cases in which the pressure of injection was so slightly increased over the normal.

Dandy and Blackfan¹² suggested in their recent communication that the mechanism of absorption was a diffuse process of the whole arachnoid. They argue an absence of function on the part of the Pacchionian granulations and hypothecate an absorption by the "exposed capillaries of the pia-arachnoid." Anatomists (cf. Mestrezat, W.) are agreed, as far as we are able to determine, that the arachnoidea is non-vascular and the pia mater is wholly lacking in a capillary bed. We have never observed in all our series any evidence of a capillary bed in the pia. Absorption into the cerebral veins coursing through the pia and finally through the arachnoid may be a possible pathway of fluid outflow, but our findings are against this view.

Under two experimental conditions we have been able to find Prussian blue granules in the cerebral veins. The first of the conditions concerns those observations in which the subarachnoid injections have been made with pressures above one hundred millimeters of mercury. In these cases granules are found throughout the walls of the cerebral veins, traversing arachnoid mesothelium, adventitia, and the inner vascular layers. Here obviously the higher pressures have been sufficient to cause the failure of the arachnoid cells and vascular layers as barriers to the injected solution. The second condition under which granular material is found in the cerebral veins deals with those altered pressure relations under which the perivascular system is injected with fluid passage into the cerebral capillaries. The cerebral veins in these cases show slight amounts of Prussian blue in

their lumina, but none in their cellular walls. This phase of the subject is considered in the following communication in this series.

(*h.*) Structure of the arachnoidal villi. — The transition of arachnoid membrane into arachnoid villus may be studied wherever villi occur. The process is similar in all cases; it will be described here for the villi in the superior longitudinal sinus, as there it is found in simplest form. In the area in which the larger cerebral veins empty into this great sagittal venous channel the arachnoidea is found undergoing this gradual transformation into the arachnoid villus. For the most part, the structure is a voluminous, delicate, and lace-like sleeve, through the axis of which a cerebral vein passes on its way to the dural channels (Figs. 1, 13), though many villi are met with which have no vascular core. The structure, however, is essentially the same whether or not the villus possesses the vessel in its midst.

In general, the villus may be described as a delicate, web-like structure of many interlacing cords, continuing the outer arachnoid membrane into the dural walls (Figs. 1, 2, 3, 5). The most marked of the villi along the great sagittal sinus in the cat and dog are those which form sheaths for the great cerebral veins, as these course over the superior longitudinal convolution (corresponding roughly to the motor area of the crucial gyrus). These villi are far more voluminous on coronal section, in the areas in which they project toward the sinus from a transverse cerebral sulcus, for here the depth of the fissures adds apparently to the length and size of the villus. This relation of the villi to the cerebral sulci becomes of anatomical and physiological importance when one considers that the chief channels of cerebro-spinal fluid are those lying in the cerebral sulci. For with the villi playing a part in absorption, as already indicated, it seems more than reasonable to suppose that they should occur in close apposition to the essential channels of cerebro-spinal fluid.

As the tubular arachnoidal villus approaches the sinus wall, the structural characteristics change gradually. The

villus, on invasion of the dural strands composing this lateral wall, changes from the interlacing strands to a solid but very delicate tissue. The basilar structure of the villus is a very fine connective tissue, reticular in structure, with the general staining qualities of myxomatous tissue elsewhere in the body (Figs. 5, 13). This delicate myxomatous reticulum is the chief characteristic of the villus as found in the animals used — cat, dog, and monkey. (In the dog, the villus may sometimes show only the strand-like morphology with almost no evidence of a myxomatous structure. This type of villus from the superior longitudinal sinus in the dog is illustrated in Figure 3. The myxomatous character is always seen in the cat, monkey, and man.) The same morphological characters are found in the arachnoid villi of infants, one of which is pictured in Figure 5. For while no Pacchionian granules are found in infants, these villi are invariably met with in normal children. This myxomatous tissue also exists as the ground-substance in the large Pacchionian granulations in the adult, and is, therefore, to a greater or smaller degree, a fundamental feature of the villus. For undoubtedly, the Pacchionian granulation must be considered as a large, hypertrophic villus, becoming evident on macroscopic examination in most adults. The more important arachnoidal villus, however, is found at all ages in man, but is not of sufficient size to be evident except on microscopic examination. The formation and significance of the Pacchionian granulation will be discussed in another communication.

Capping on all sides the myxomatous basilar structure of the villus is the mesothelial covering of arachnoid cells. These serve to keep intact the structural characteristics of the arachnoid projection, and in places to act as the cellular filter into the great sinuses. Ordinarily, the cells about the villus form a thin layer, but in other areas the greater part of the arachnoid tuft appears to be composed of these cells. In Figure 13 is shown a cellular cap of a villus approaching the lateral wall of the sagittal sinus. This increase in the cellular elements is seen in all of the

arachnoid villi which project into the cavernous sinus (Fig. 7). Such a cellular structure renders easy the identification of a basilar type of tuft in contradistinction to the tegmental or sagittal variety. This redundancy of cells gives source to many peculiar and, at times, confusing pictures.

The general shape of the arachnoid villus in the different animals used varies considerably, the variations being dependent on the age and perhaps on the individual. In cats and dogs the sagittal villus, in characteristic cases, is chiefly confined to the lateral wall of the sinus, except in the area in which the great cerebral veins enter the superior sinus. In this area occur large projections of the arachnoid tissue into the sinus (Figs. 3, 5, 13). These are irregularly shaped, warty excrescences, sessile or pedunculated. They are met with in coronal sections which show them apparently lying free in the lumen; they are often attached to the superior wall of the sinus; or, again, they may have only the inferior area of the sinus triangle to fill. For the most part they show the basilar reticulum of myxomatous tissue (Figs. 5 and 13), but in some cases these intraluminal structures maintain the strand-like character of the villus as it begins to leave the cerebral surface (Fig. 3). In monkeys and in man the same general structures are found invading the dural wall to project into the sinus; they are ordinarily microscopic in size, but those in adult human beings become enlarged so that they are evident on macroscopic examination. The villi in the anthropoideæ and in man are very numerous in the irregular diverticula of the superior longitudinal sinus—the lacunæ laterales—in the motor area (Fig. 13). This same phenomenon of arachnoid invasion lateral to the main lumen of this sinus has been met with in one case in a dog in which the villus approached a cerebral vein in its short dural course to the great sinus.

The passage of fluid from these arachnoidal villi into the sinus may be studied only when a means of precipitating in situ the injection fluid is possible. In these observations made after low pressure injections or after the replacement of cerebro-spinal fluid with a ferrocyanide solution, the fluids

may be traced by the precipitated granules from the subarachnoid spaces into the lumina of the sinuses. As recorded in the foregoing communication such a fluid passage may not take place from the arachnoid villus as it first approaches the lumen of the sinus, but it occurs in the more posterior portions when the villus may be no longer apparent and the precipitated granules appear solely in masses of arachnoid cells which are in closest apposition to the endothelium of the sinus. These granular collections, as well as the cells in which and about which they lie, can always be traced in serial sections back to the original arachnoid villus. Such phenomena are apparent in Figure 3, in which a large villus is shown occupying the lateral wall of the sinus. No passage of granules from this cord-like villus is made out, but in a lateral diverticulum of the sinus the passage of granules from an arachnoid cell-collection is apparent (Fig. 4). The villus then may be looked upon, in part, as a mechanism for the passage of fluid into the arachnoid cells which cover it and eventually serve to pass fluid into the great sinuses. Owing to the close relations which exist between the tubular villus and the mesothelial cell-collections, we have come to employ the term "arachnoid villus" for both the tubular structure which projects toward the sinus from the arachnoid membrane and the cellular cap of cells which lie in close approximation to the sinus lumen.

(*i.*) The arachnoid cell-nests. — Projecting from the arachnoid membrane itself, or from the cellular portion of the villus, peculiar collections of arachnoid mesothelial cells are found in almost every part of the dura, especially in the vicinity of the great sagittal and of the cavernous sinuses. In the cat and dog there appears to be far less tendency on the part of the arachnoidal mesothelial covering to form such cell-clusters or nests (except in the basilar tufts), but in man and monkey also many of the cell congregations are found. These arachnoidal cell-islands stand out clearly from the surrounding dural tissue (Figs. 4, 12); for the pachymeningeal structure is that of interlacing bands of dense

fibrous tissue with very little interfibrillar supporting substance. The nuclei seen in the dural strands are few in number and are all elongated spindles. On the other hand, the arachnoidal cell-collections within the dura exhibit a clear differentiation from these dural strands; the cytoplasm is rather scant, the nuclei are round or slightly oval, large, and rather deeply stained with hemotoxylin. In their customary clusters they look not unlike the cells found in many of the mesodermal tumors. The occurrence and significance of these arachnoidal cell-nests have been commented upon by Ribbert,⁴³ by M. B. Schmidt,⁴⁶ and more recently by Wojna.⁵² Schmidt connects these cellular accumulations with the origin of the so-called "dural endotheliomata" — a conception which has been fully substantiated by observations, as yet unpublished by Cushing. Wojna offers evidence that these villi with the arachnoidal cell-clusters are the points of true cerebral herniation.

Proof that these cell-nests are arachnoidal in origin depends on several observations. In the first place, by serial sections, practically all of these cellular collections can be traced back to the original mesothelial covering of the arachnoidea. On spinal subarachnoid injection in the living animal with the ferrocyanide solution these cellular columns, for the most part, show between the cells the typical Prussian blue precipitate, whereas the surrounding dural strands are entirely lacking in the granules (Fig. 12). As to the few cell-nests which seem to be isolated entirely from arachnoid tissue the cellular structure and general appearance of the cells coincide exactly with those of the cellular collections which may cover the arachnoid villus. It seems likely from the results of these ferrocyanide injections and from the structural relations that these arachnoidal cell-masses serve as intra-dural channels for cerebro-spinal fluid.

Further evidence of such an idea of circulation of cerebro-spinal fluid through the pachymeninx is obtained from observation of the bleeding from the outer surface of the dura, after elevating or removing the calvarium. In these

cases, the blood which oozes from this exposed meningeal face is very thin and watery, showing an apparent mixture with some thinner fluid than blood. In some cases, also, punctate areas of exudation of watery fluid unmixed by blood are observed. These undoubtedly correspond to the underlying arachnoid channels. The fluid exudation is undoubtedly cerebro-spinal fluid, and, in no sense, lymph.

In addition, interesting phases of this exudation of cerebro-spinal fluid through the dura appear in both the ferrocyanide and carbon granule injections at high pressure. If, in one of these preparations made by injecting into the subarachnoid space a trephine opening be made and the dura left undisturbed, fluid, at first only in punctiform distribution, will be seen to exude from the exposed pachymeninx. In the case of the ferrocyanide preparation the dura is found, on microscopic examination, to be deeply stained with the Prussian blue; granules are found throughout the fibrous tissue in diffuse distribution. The outer layer of arachnoid mesothelium, lying beneath the trephine opening, is likewise filled with the granular precipitate, while similarly the subdural space in this area shows the ferrocyanide deposit. On the other hand, with the carbon granule suspensions, colorless, clear fluid exudes from the exposed dura, but neither the arachnoid membrane, the subdural space, or the dura itself, show the granules. This observation undoubtedly indicates that the arachnoid and the dura both become permeable to fluids, if the pressure relations are such as to facilitate the passage of fluid. Evidently the process must be one of filtration, for otherwise the passage of carbon granules through the membranes must have occurred. This same phenomenon of fluid passage through the arachnoid and dura occurs when in the course of fixation of the whole head in forty per cent formalin containing acid a portion of the skull cap is removed before the acid has precipitated the Prussian blue. Evidently here the process is one of osmosis and diffusion with the injection mass with salt content on one side of the membrane, and the acid formalin solution on the other. This results in the formalin passing to the solution

of higher salt content (osmosis) with the salts passing to the formalin solution (diffusion).

These two phenomena are probably due to different causes than the "bleeding" of cerebro-spinal fluid, which occurs from the dura in those cases in which the subarachnoid tension is normal. For in this latter series the punctiform distribution of the "bleeding," the presence of cerebro-spinal fluid channels in the dura, and the lack of abnormal pressure relations all argue for the leaking of cerebro-spinal fluid from the arachnoidal cell-columns. Probably many of the arachnoidal columns on their way into the diploe are broken off when the skull cap is raised. Such a view accounts very well for the punctate distribution of the fluid.

(*j.*) Method of fluid escape from the cerebro-spinal spaces.—With such a general conception of the process of fluid conduction within a membrane covered and lined by mesothelium, the question of fluid escape from the membrane has to be considered. In an earlier paragraph it was stated that the fluid could be traced, by the precipitated granules, through the endothelial cells into the sinus (Fig. 4). In the cat and dog it has been very difficult to determine whether there is a double layer of endothelial cells to traverse—a layer composed of arachnoid mesothelium and vascular endothelium. As Key and Retzius first pointed out, such a double arrangement of these mesodermal cells is evident in the Pacchionian granulation in the human being, but when one studies the much more delicate villi in the lower animals, slight uncertainty exists. However, we believe from histological evidence and from the evidence afforded by subdural injections, that these double layers of mesodermal cells characterize all the arachnoid projections which have been seen. Undoubtedly this complete covering of the villi with mesothelium is continued to all the lower forms which have this method of drainage.

In the study of all the mesodermal cells which exist between the myxomatous ground-work of the villus and the lumen of the sinus, it becomes evident that the escape of

fluid from the villi is probably through the cell-substance, and probably not between the cells as Key and Retzius suppose to be the case for the Pacchionian granulations. (The observations of Key and Retzius in regard to the mechanism of fluid passage into the sinus are, as already pointed out, of little value as their gelatine masses ruptured the wall of the Pacchionian granulation in all probability and thus permitted the injection mass to pass into the venous channel.) The granules are found between the mesodermal cells and also within their cytoplasm in the areas in which the dural strands are lacking between the villus and the lumen of the sinus. It is extremely difficult to exclude the possibility of fluid passage into the sinus between the cells, and in many areas the escape of fluid seems to be both through and between the mesodermal cells (Fig. 4). On theoretical grounds the passage of fluid through a living cellular membrane, which is probably a passage through cells, seems most likely in this problem. Hence we may assume the mode of escape of cerebro-spinal fluid to be a passage through the external covering of the villus similar to the passage of fluids through any semi-permeable membrane. In addition to the histological evidence of such a filtration by a membrane as afforded by the precipitation of the Prussian blue granules from the true solution, the failure of carbon and other granular suspensions to pass through lends support to the view. Certainly there is no histological evidence for actual stomata, but all the data favor the idea of a filtration by a semi-permeable membrane. And the proof of such a passage must be had from observations upon cells which were living at the time of fluid passage and not after the death of these cells. On the inner surface of the endothelial lining of the sinus the characteristic granules of ferric ferrocyanide can be easily seen in these areas of fluid absorption — supporting evidence, when taken in conjunction with the finding of the granules throughout the cell-bodies, that the fluid normally enters the venous circulation in this manner. Furthermore, in some cases, the granules are found in the coagulated blood in the sinus — a finding quite striking when one considers that the injections

were made in the living animal with the blood coursing through the sinus. (In many instances we have confirmed Lewandowsky's finding that after intraspinal injection of the ferrocyanide the salt may be detected in the urine in twenty minutes. We have not taken samples of the venous blood from the head as was done by Ziegler and by Reiner and Schnitzler.)

The process, then, of fluid escape from the cerebro-spinal spaces is one of passage from the arachnoidal villus through a mesodermal membrane into the great sinus. And when one speaks of fluid passage through a cellular membrane, the factors of osmosis, diffusion, and filtration must be considered. Assuming osmosis to be the power of concentrated fluids to attract water or fluid from less concentrated solutions, it is not difficult to explain the normal physiological process of absorption of cerebro-spinal fluid on this basis; for the venous blood certainly possesses a greater concentration in colloids and crystalloids than the cerebro-spinal fluid which is as poor in salts as any of the body fluids. This greater concentration of the blood would naturally attract fluid from the arachnoid villus into the sinus. A similar argument has been advanced by Mott³⁶ in support of his theory of capillary absorption of cerebro-spinal fluid. Diffusion would, of course, cover this passage of fluid from one side to the other of the membrane. But finally, when one conceives the conditions of cerebro-spinal secretion, it seems not unlikely that the "vis a tergo" of the fluid is responsible for its passage into the sinus by the simple process of filtration. It is generally conceded that the choroid plexuses elaborate a certain amount of cerebro-spinal fluid in the course of twenty-four hours, and this requires a proportionate flow through the subarachnoid channels. The mechanism of the cerebral pressure relations is such that the cerebro-spinal fluid pressure is continually being reduced to that of the venous circulation, and, in consequence, there must be a constant absorption of the fluid to compensate for the constant secretion. In all probability, the pressure of secretion is considerably higher than that of the fluid at the point of venous absorption—this difference is necessary to overcome the

resistance offered by the complex bed through which it flows. The pressure in the arachnoid villi undoubtedly remains constantly at a slightly higher level than that in the sinus, so that in order to maintain the equality in pressure and to preclude an increase in subarachnoid tension from the continued elaboration of the fluid the cerebro-spinal fluid filters into the sinus through the mesodermal cells.

With such a conception of the process of absorption through a mesodermal membrane it is not difficult to understand the variability of results which are obtained by the injection of granular substances and of viscous fluids. The earlier injections which we made with suspensions of carbon granules revealed an occasional granule in the cytoplasm of the endothelial cells of the sinus, but these were apparently chance findings and no real index of function. On the other hand, the low pressure injections with the ferrocyanide solution resulted, after precipitation *in situ* and fixation, in a general distribution of the granules throughout the myxomatous ground substance of the villus and a great transit of the granules through the cells. This tremendous passage of the solution as evidenced by the precipitated granules argues for the filtration mechanism as the essential factor in absorption, for the pressure of injection was so slightly above the normal subarachnoid tension. The factors of osmosis and diffusion would be less potent in the case of injections of ferrocyanide solution which contained these salts in higher concentration, for on this basis the flow should be from blood vessel to cerebro-spinal space. Yet the results of these injections with concentrated solutions are identical with those with solutions practically isotonic with the blood.

The whole question, then, of cerebro-spinal fluid drainage may be taken as one of filtration of the fluid through the arachnoid meshwork and through a mesodermal membrane. Whether osmosis and diffusion play a part in the process cannot be decided without further evidence; the evidence at hand permits only the statement that increased pressure of fluid on the arachnoid side of the membrane permits fluid to pass into the sinus.

(*k.*) Retrograde passage of fluid through the arachnoid villus. — With this conception of a filtration of cerebro-spinal fluid the question arises as to whether fluids ever pass from the sinus into the cerebro-spinal spaces. This is an important matter and one which also permits of experimental approach. The one factor of error in the solution of this question is the possible permeability of the choroid plexus which might vitiate any evidence of a retrograde (?) passage through the arachnoid villus.

The first series of observations carried on in this phase of the study of cerebro-spinal fluid drainage dealt with vascular congestions. It was felt that a more complete and probably more normal distension of all cerebral blood vessels could be secured in this way than by any other. Hence, in anesthetized cats and dogs the two carotid arteries, the two vagi, and the trachea were dissected out and a tourniquet applied tightly about the other structures in the neck. On tightening the tourniquet the animal was deprived of any return from the cerebral vessels except through the veins of the spinal canal. With the onset of asphyxia the arterial pressure was raised considerably, thus ensuring a more extensive injection of the veins. After death of the animal the whole head was fixed in situ in forty per cent formaldehyde. On removal of the hardened brain enclosed in meninges no macroscopic evidence of diffusion of blood into the subarachnoid space was disclosed. The cerebral vessels were all, however, greatly engorged, and even the small vessels were easily made out. On microscopic examination, in all but one case, there was entire absence of any red blood corpuscles in the subdural and subarachnoid spaces, even in the area of the arachnoid villi. In the one exception there were a few corpuscles in the subdural space in close apposition to the superior longitudinal sinus and also a few corpuscles caught in the meshes of the arachnoid villi which entered the sinus. Whether the corpuscles had entered these meningeal spaces by diapedesis or by rupture of some essential element in the sinus wall, could not be determined. The rupture, if existent, was not apparent on careful microscopic examination. That the

corpuscles did not enter the meningeal spaces from the choroid plexus was evidenced by the fact that no corpuscles were found in the subarachnoid spaces over the hemispheres, by the fact that corpuscles were found only in the meningeal spaces in close approximation to the sinuses, and by the fact that the ventricles of the brain gave no evidence of vascular diffusion. Just how to account for the fact that in one case we were able to find erythrocytes in the meningeal spaces we do not know. Whether this one particular animal possessed a particularly high blood pressure or a weak arachnoid membrane cannot be told. It seems very likely that the trauma necessarily inflicted upon the dura in removing the calvarium might have occasioned the few corpuscles to leave the congested sinus or the distended cerebral veins just before they empty into the sinus. It is safe to assume that under ordinary circumstances, however, corpuscular forms or granules do not pass into the subarachnoid spaces even when the venous pressure is raised to that of the arteries.

Has the plasma left the vascular bed and passed into the cerebro-spinal spaces to compensate for this tremendous increase in venous and consequently in intracranial pressure? It must be realized in this consideration that we are dealing with a cavity which is always filled with fluid and which is almost non-distensible. With a slight degree of distension possible in the system it may be considered in the initial stages of such an experiment that the venous pressure is momentarily higher than that in the cerebro-spinal space, permitting, therefore, some transudation as a compensatory phenomenon. This question of fluid passage in a reverse direction can only be answered by the application of certain analogous observations. It has been found that if a ferrocyanide solution, under a pressure of forty millimeters of mercury, be injected into the jugular vein (after washing out the blood), there is macroscopic evidence of the blue precipitate in the meningeal spaces about the superior longitudinal sinus. On microscopic examination the precipitated granules of Prussian blue were evident in the arachnoid villi and in the subarachnoid spaces for some distance from the

longitudinal sinus. No granules were found in the ventricular cavities nor in the subarachnoid spaces over the convexities except as already described. When, however, the closed cavity factors are eliminated by opening the cerebro-spinal spaces (as by cistern puncture) an extensive passage of solutions from the sinuses through the villi take place.

These two series of observations afford evidence for the view that the sinuses can yield fluid contents to the subarachnoid spaces — a mechanism for the maintenance of a disturbed pressure equilibrium between the two sides of the apparently semipermeable membrane of the villus. Whether this process goes on normally or not is another question, and this evidence in no way permits us to speak of this function. As far as can be made out there appears to be but little support to any theory of a retrograde passage of formed elements. Certainly there is no evidence of a valve-action in the process of drainage unless the pressures used in these injections were such as to occasion rupture of valves, which cannot be identified morphologically.

(L.) The distribution of the arachnoid villi. — This has not occupied our attention very greatly as it has been felt that the essential part of our problem was to work out as far as possible the pathway of fluid drainage in one area in the cerebro-spinal axis. Undoubtedly, the same process occurs elsewhere over the meninges wherever the villi are functioning. Key and Retzius²⁴ reported that the Pacchionian granulations occur in the following situations in order of frequency — superior longitudinal sinus, transverse sinus, cavernous sinus, superior petrosal sinus, and *venæ meningiæ mediæ*. Our work has been chiefly on the lower animals and, in consequence, our data deals with the occurrence of arachnoid villi rather than with what we regard as pathological structures which arise from them, viz.: the granulation. Those which we have studied chiefly have been projecting from the leptomeninges into the lateral wall of the great sagittal sinus. We have also been interested in the occurrence of constant arachnoid villi invading the cavernous

sinus in the cat and dog (Fig. 7). In addition we have found arachnoid tufts in the inferior longitudinal sinus and in the transverse sinuses—the process is everywhere the same except that the tufts are much smaller and are composed of more mesothelial cells and less myxomatous tissue than are those in the sagittal area of absorption.

(*m.*) Absorption from the spinal and cranial portions of the subarachnoid space. — We have attempted in a series of observations to ascertain the rates of absorption from various areas in the cerebro-spinal axis. In an intact system, saline solution introduced by a cannula in the caudal end of the spinal subarachnoid space was absorbed at a rate three times more rapid than when the cranial mechanism was excluded by ligation of the cord in the lower cervical region. This three to one ratio we found to hold for constant pressures ranging from two hundred to six hundred millimeters of water. With pressures of thirty to fifty millimeters of mercury the ratio drops somewhat.

As mentioned in an earlier paragraph we have repeatedly confirmed Lewandowsky's observation that after introduction of potassium ferrocyanide into the subarachnoid space it may be detected in the urine in about twenty minutes. When, however, the solution is introduced in the isolated spinal subarachnoid space it appears in the urine only after seventy-five minutes and then only in very minute traces.

Dandy and Blackfan¹² found a rate of absorption for phenolsulphonaphthalein proportionately as great from the spinal subarachnoid spaces isolated from the cranium as with the whole axis intact. We have made several series of observations with the intraspinal absorption of phenolsulphonaphthalein. In two hours in cats we find a fairly uniform excretion of this dyestuff averaging between nineteen and twenty-five per cent from the intact spinal subarachnoid spaces. When, however, the spinal cord and meninges are ligated off in the lower cervical region the dyestuff introduced in the spinal spaces caudal to the ligation is usually excreted in the urine in percentages for the

same period below ten, the highest recorded reading being eleven per cent.

The more rapid absorption with the cranial system functioning indicates a far greater power of drainage than is present in the spinal cord. Such a conclusion is based on the fact that no fluid is secreted into such an isolated spinal space to any extent, whereas with the cranial mechanism intact the whole flow from the choroid plexus is added to the amount of the fluid to be absorbed. Hence in all probability the cerebral pathways drain many times the amount of fluid which can be cared for by the spinal pathways, and many times more than the actual percentages indicate. For unless one assumes that the absorption of phenolsulphonaphthalein is a selective function on the part of the membranes it follows that its percentage of absorption and subsequent renal excretion are directly proportionate to the amount of absorption of the fluid in which it is diluted.

In our observations with the rate of absorption we have used cats anesthetized with urethane. In general, with the ferrocyanide and with the phenolsulphonaphthalein we have withdrawn one cubic centimeter of spinal fluid and immediately replaced it with an equal amount of the foreign solution.

The technical difficulties attending this replacement of one cubic centimeter of cerebro-spinal fluid from the isolated spinal subarachnoid space are great in the lower animals. The cord must be ligated in the lower cervical or upper thoracic region before the fluid is withdrawn and there must be no leakage around the needle. Using cats and small dogs we have never felt that the pressure in the isolated space was not increased by the procedure. Hence we have with the same dyestuff attempted the solution of the problem in another way. It was found that if in anesthetized cats one cubic centimeter of cerebro-spinal fluid be withdrawn from the cerebellar cisterns through the occipito-atlantoid ligament and an equal quantity of phenolsulphonaphthalein be immediately introduced, the dyestuff was present in the urine after two hours in constant percentages of twenty-four

to twenty-six. If a similar procedure be carried out with preliminary ligature of the cord in the upper thoracic region, the renal secretion is not changed at all — the two types of experimental animal excreting the twenty-five per cent proportion. Ligature of the cord itself affects the excretion of the dyestuff but little — our controls showing a rather low normal percentage after intramuscular injection.

Such considerations and such observations make it apparent that normally the spinal subarachnoid space plays but little part in the normal absorption of cerebro-spinal fluid, for with it isolated, the cranial absorption rate is unaffected. Hence this physiological evidence supports strongly the idea of a major cranial absorption.

(*n.*) A potential subdural space about the arachnoid villus. — With the evidence presented thus far for a cerebro-spinal drainage by a process of filtration through the membrane covering the arachnoid tuft, it seems fitting to consider Key and Retzius' conception of the Pacchionian granulation as being surrounded by a potential prolongation of the subdural space. Throughout our description of the mechanism of absorption we have dealt with the subdural envelope as a potential rather than an actual space. In the ordinary process of absorption, fluid passes, according to our interpretation of the evidence at hand, directly from the arachnoid tuft through a mesodermal membrane into the sinuses. That Key and Retzius were able to inject, by simultaneous subdural and subarachnoid solutions, a subdural space about the granulation, really plays but little part in our considerations of the process. This conclusion has been based on a series of observations which we have made. No evidence of continuity between spinal subarachnoid and cerebral subdural spaces is afforded by Key and Retzius' injections of gelatine solutions, and the same is true of our injections of carbon granules, or of the ferrocyanide solution — when made under low pressures. We have made many observations in which the pressures have been considerably elevated. With slow injection under high pressure

(100 mm. Hg.) with both a ferrocyanide solution and an india ink suspension a few granules may be found in the subdural space. In these preparations there is considerable evidence that the fluid and the granules passed through the mesothelial cells covering the arachnoid membrane.

When, however, the subarachnoid injections were made under very high pressure (150 mm. Hg.) the subdural collections of granules became very markedly accentuated. The subdural space was distended with the carbon suspensions, while the subarachnoid was practically collapsed. The study of the arachnoid villi in these cases proved to be instructive. The chain of carbon granules penetrated between the arachnoid villus and the dense strands of dura, apparently breaking the delicate structures which approximated villus to dura, and, finally, came to lie just beneath endothelium in the wall of the sinus. In this situation the carbon granules occupied the area that the arachnoid mesh usually held, and they undoubtedly entered the sinus as they could be found in the lumen. (The finding of the granules of carbon in the lumen of the sinus should be expected in this case as the high pressures used were sufficient to kill the animal through paralysis of the respiratory center. Under these circumstances, with no blood flowing through the sinus, any granules which passed into it should remain practically in situ.) This finding, we believe, is of significance when one considers the former doubt as to the continuity of the spinal subarachnoid space with the cerebral subdural space. Either connection desired may be obtained, the result being determined solely by the pressure of injection in the spinal region. For in the injections at high pressure the arachnoid membrane apparently loses its integrity and allows the injection mass to enter the potential subdural space. When the pressure is increased greatly there is apparently a rupture of the arachnoidea and the whole injection fluid passes subdurally. This is especially true in the case of the granular injections with india ink suspensions, due, in our opinion, to the fact that there is a mechanism of filtration in the villus, the granules being held back while there is but little resistance

to fluid drainage. We have seen only a very small amount of Prussian blue precipitate in the subdural space even with these very high pressure injections, but with the granular injection masses there have been enormous numbers of the subdural granules. Hence, the tendency to rupture after an injection of granular material is much greater than with fluid solutions. This finding undoubtedly is in accord with the subdural injections made by Key and Retzius, for in these cases their masses displaced the villus from its dural bed as was noticed in our high pressure injections, which resulted in subdural distribution of the masses.

This process of escape from the subdural space may be a normal mechanism, or, at least, an adaptive one, available in those cases in which collections of fluid occur subdurally. It accounts very well for the drainage of mercury, gas bubbles and fluid under pressure, from the subdural space in the earlier observations of Cushing. Likewise, it furnishes evidence regarding all of the processes of cerebro-spinal drainage and makes it seem evident that care must be taken to avoid excessive pressures in injection studies, for entirely different pictures can be obtained by varying pressures.

(*v.*) General consideration of the escape of cerebro-spinal fluid into the venous sinuses.—The mechanism of absorption, then, of cerebro-spinal fluid concerns a passage of the fluid through the mesodermal cell-membranes of the arachnoid villi into the lumina of the great sinuses. Our results indicate that the process is one of filtration from the point of higher pressure to that of lower pressure. Our observations coincide in the main with those of Key and Retzius, in so far as they assume the Pacchionian granulation to be concerned in the process, but with the modification that the normal mechanism deals with an arachnoid villus and not with the abnormal Pacchionian transformation of a villus. We believe that the process of drainage is through a cellular membrane and not between cells (*i.e.*, through stomata or stigmata) in the view of Key and Retzius.

With such a histologically delicate pathway as we have

described one may reconcile the findings of earlier writers with our present views. For instance, the rupture of the rubber bag containing mercury (Cushing) into the subdural space was sufficiently abnormal to vitiate the results. The mercury could reach the venous circulation by rupture of the villus or by stripping the villus away from dura, as happened in our high pressure india ink injections. Mercury is altogether too heavy a material for use as injection mass, although it has the advantage of being fluid, and, in consequence, of passing probably without interruption through the arachnoid filter. Non-absorbable gases, likewise, can probably pass without trouble through the mesh-work. The findings of Quincke, of Reiner and Schnitzler, of Spina, of Ziegler, of Hill, and of Lewandowsky, all accord with our view of the escape of cerebro-spinal fluid. The conception of this process, advanced by Goldmann, by Dandy and Blackfan, is at variance; their ideas find but little support in our observations. Apparently the mechanism of absorption does not concern a valve-like process except in so far as the mesodermal cells possibly may in themselves act as valves, allowing fluids to pass through them in one direction, far more easily than in the reverse direction. There is but little support for this view, not enough to permit of more than a suggestion. Our evidence inclines strongly to the view that there is a simple process of membrane filtration concerned in the absorption of cerebro-spinal fluid.

II. THE ACCESSORY OR LYMPHATIC PATHWAY OF ABSORPTION.

In addition to the chief or venous pathway of absorption of cerebro-spinal fluid there exists an accessory mechanism of drainage through the lymphatic system. This was demonstrated first by Key and Retzius,²⁴ and later by Hill.²¹ The former were able to inject with colored gelatine masses the cervical lymphatics from the spinal subarachnoid space. Hill found, after subarachnoid introduction of saline solution

colored with methylene blue, staining of the cervical lymph glands after one hour, whereas after a very few minutes the dyestuff was detected in the bladder and in the stomach. Cushing¹¹ found no evidence of an accessory pathway of drainage when he used non-absorbable gases and mercury. Cathelin⁷ strongly argues for an exclusive lymphatic drainage of the fluid, being inclined to disbelieve all the evidence of absorption into the venous system. Dandy and Blackfan,¹² with phenolsulphonephthalein, show a small and greatly delayed drainage of the dyestuff into the thoracic duct. The evidence to date then shows a lack of unanimity regarding the exact absorption of cerebro-spinal fluid through the lymphatic system.

(a.) Gross findings in the accessory pathway.—Our earlier observations, made after injections of suspensions of carbon granules into the spinal subarachnoid space, under pressures slightly above the normal and over considerable periods of time, led us to the tentative conclusion that such a lymphatic drainage did not exist for these granular substances. For in these earlier cases, even in those in which the carbon granules were massed about the basilar structures and in which the perineural injections were outspoken, no granules could be found on microscopic study in the cervical lymph glands and their channels. However, when the ferrocyanide solution is substituted for the carbon suspension, and the pressure conditions maintained at the same level, slightly above normal, an entirely different picture of the cervical region is obtained. In these cases, with fixation of the whole head by plunging into forty per cent formaldehyde or by carotid injection of ten per cent formalin (both solutions being acidified by hydrochloric acid), the upper neck structures are preserved in situ. The acid secures precipitation of the ferric ferrocyanide and the pathway of lymphatic absorption can be worked out in the neck by application of the same methods as proved of value in the cranial chamber. On dissection of the neck after such a preparation, blue cords are easily made out, running in the carotid sheath in

the lower part of the neck and also in other situations higher in the neck. Carrying the dissection upward, lymph nodes become visible, intensely colored with the Prussian blue. The most frequent gland to show the injection mass is the large cervical node which lies directly beneath the angle of the jaw in these lower animals. More careful dissection enables one to separate out the different colored cords and to identify them. In every case they proved to be either lymphatic channels or cranial nerves, the granules in these latter cases being perineural. The same type of perineural injection was found in all the preparations, especially about the more cephalic of the cranial nerves. And as our evidence in these cases indicates that this perineural pathway is merely a stage in the mechanism of lymphatic drainage it should be discussed and described as a phase of the accessory system of absorption for cerebro-spinal fluid.

The extension of the arachnoid membrane for short distances out along the cranial and spinal nerves has been generally accepted for many years. This arachnoidal prolongation is described as ending in a cul-de-sac, over which the dura is continued for a short distance peripherally. But in addition to this evident meningeal sac there occurs around each nerve a perineural space which is capable of injection under varying pressures from the subarachnoid space. Cotugno,¹⁰ to whom credit for the discovery of cerebro-spinal fluid is generally given, was the first to demonstrate this perineural space by injecting it with mercury and with air. Key and Retzius also demonstrated potential spaces along the cranial nerves in their gelatine preparations. And in our observations with different methods of injection we have been repeatedly able to confirm the finding, especially in the cases in which we have made use of the ferrocyanide solution.

(*b.*) Cerebro-spinal spaces along the olfactory nerves. — The first pair of cranial nerves, the olfactory, customarily shows marked deposits of Prussian blue in the perineural spaces, following an injection of the ferrocyanide solution into the spinal subarachnoid space. The great masses of

the granules in the arachnoid cul-de-sacs just above the cribriform plate were striking, but the coloring passed directly through the foramina in the plate into the walls of the nasal cavity. This resulted in an apparently diffuse staining of the mucous lining of the nares, the blue color being very dense and uniform on macroscopic examination. When examined microscopically, very peculiar pictures in this nasal mucosa are seen. The granular material seems to lie chiefly beneath the epithelial cells of the mucous surface in the meshes of a loose connective tissue (Fig. 8). The granules are found adhering to the fiber strands but are not included in any cellular structures. In the midst of this loose reticulum occur wide vessels lined by endothelial cells, but with practically no supporting connective tissue in the shape of media or adventitia. These are large, very thin-walled channels, lymphatic in nature (Fig. 8). The belief that these vessels are lymphatic in nature is founded chiefly on the fact that wholly similar channels may be filled by a lymphatic injection beneath the nasal membrane. In addition, there is the confirmatory evidence of the character of the walls of these vessels compared with the size, the absence in them of red blood corpuscles, and the proximity of other venous channels readily identified by corpuscular content. The relation of these wide vessels to the Prussian blue granules in the loose connective tissue is fairly definite. The granules may be identified in the walls of the vessels apparently passing into the channel, for they are found also in the endothelial cells lining the vessel and free in the lumen.

This finding of the injection fluid passing from the connective tissue reticulum into these wide lymphatic vessels is, we feel, of importance in the solution of the accessory pathway. For if the granular precipitate truly represents the course taken by the original injection fluid, certainly some of the fluid must have passed from the nasal connective tissue into these wide vessels. But how does the fluid first reach this reticular structure? This question can be answered by study of the olfactory area of the nasal membrane. In this limited

field the large olfactory nerves (on, Fig. 8) can be easily made out coursing through the basilar connective tissue structures. Surrounding these nerves are definite collections of ferrocyanide precipitate, but these collections become more diffuse as the nerves approach the membrane, and finally the granular deposit is found almost entirely in the surrounding tissue network. This indicates a discharge of the injection fluid into the nasal membrane, from which it is absorbed into the wide lymphatic channels. In other areas the connection between perineural space and lymphatic stream is much closer than in the observations just recorded, but apparently in every case the absorption from perineural space by lymphatics is through the agency of the tissue spaces intervening and is in no way direct.

Certain interesting phases of the pathway of cerebro-spinal fluid escape along the olfactory nerves became noticeable in some of the observations. In those cases in which the ferrocyanide mixture was introduced into the spinal subarachnoid space under very high pressure, fluid was observed to drip from the nose of the animal. Tests of this nasal fluid showed it to be the ferrocyanide solution. This indicated the possibility of causing a cerebro-spinal rhinorrhea by mere increase of the cerebro-spinal pressure; in these cases the epithelial lining of the nose apparently became permeable to the fluid under the higher pressure. Spina has reported a similar escape of a fuchsin solution from the nose after intraspinal injections under high pressures and with depressed head.

A similar ferrocyanide cerebro-spinal rhinorrhea occurred in one case in which, after spinal subarachnoid injection of the ferrocyanide solution, acid formalin under pressure (of 100 mm. Hg.) was injected into the carotid artery. A mass ligature had been placed in this animal about all of the neck structures. After the formalin had run into the artery for a few moments, fluid, colored with the characteristic Prussian blue precipitate, came from the nose. The correct explanation of this phenomenon doubtless lies in the fact that under the experimental conditions the pressure in the

cerebro-spinal spaces was raised to the level of the arterial and venous tensions (*i.e.*, 100 mm. Hg.). This high subarachnoid tension forced fluid out through every possible way of escape with the development of the rhinorrhea. The precipitation of the ferrocyanide must have occurred at the time of passage through the nasal membrane, due to the presence of the acid formalin in all the capillaries.

When low-pressure injections of granular materials of several hours' duration are made into the spinal subarachnoid space it has proved impossible to secure injection of the olfactory nerves beyond the cribriform plate. It seems probable that it is possible to inject only the prolongations of the arachnoid membrane along the cranial nerves with these suspensions, while the finer perineural space remains unfilled by the granular mass. The distribution of carbon granules in those cases of high pressure injection is somewhat different, in that a few granules can be made out in the reticular tissue beneath the nasal epithelium. Here again is an illustration of the difference in findings dependent on pressures at which the injections were made. For these higher pressures doubtless break down the delicate membrane which serves as a fluid retainer to maintain intact the cerebro-spinal spaces.

(*c.*) The fluid spaces about the optic nerve. — Both the carbon suspensions and the ferrocyanide solutions when injected into the spinal subarachnoid space caused collection of the characteristic granules along the subarachnoid sheath (infravaginal space) of the optic nerve. The injection masses passed out along the nerves in the subarachnoid spaces and spread out over the posterior surface of the eyeball (Key and Retzius,²⁴ Schwalbe⁴⁷). The ferrocyanide solution gave a much wider area of spread over the globe than the carbon granule suspension, probably due to the retention of the granules by tissue networks.

On section of the eyes in the case of a subarachnoid ferrocyanide injection the characteristic granules could be identified in the epichoroidal and in the episcleral space of

Schwalbe. This investigator believed the space to be connected with the cranial subdural space, but Key and Retzius showed the error of this assumption. The fluid passage from cerebral subarachnoid space to the ocular episcleral space is by way of the optic subarachnoid, the optic subdural into Tenon's capsule (Schwalbe believed this to be a direct continuation of subdural space with partial interruption around the second nerve insertion). The granules can be traced around the bulb toward the filtration angle in this space and the correspondence of the two processes — drainage of the ocular humors and of the cerebro-spinal fluid — becomes marked. This correspondence between the two processes will be more fully discussed in a later communication.

In this connection the reports of Albert and Schnitzler¹ are of interest. These observers, studying the rate of absorption of cerebro-spinal fluid under varying pressures, noted a marked bulging of the sheaths of the optic nerves when the intracranial tension was greatly increased.

(*d.*) The perineural spaces of other cranial nerves. — The findings along the other cranial nerves after spinal subarachnoid injections are quite similar, in the gross principle, to those already detailed. All of the nerves show deposits of Prussian blue upon them as they leave the dural envelope, and for varying distances the injection mass can be traced along them. Eventually, the granules come to lie in the perineural spaces just beneath the epineurilemma. This phenomenon is particularly well shown in the case of the caudal three cranial nerves. The vagus after a routine spinal injection of the ferrocyanide solution shows the blue precipitate usually as far down as the middle of the neck and, in exceptional cases, to the episternal notch.

The spinal accessory also, in these typical preparations, exhibits a granular collection for one or two centimeters from the base of the skull. In the case of the twelfth nerve it is not unusual to find the perineural injection passing a considerable distance toward the base of the tongue and, in

one exceptional injection, the whole major extent of the nerve showed the Prussian blue precipitate beneath the epineurilemma. This finding Key and Retzius have already made with their gelatine injections. On clearing the petrous portion of the temporal bone, or on dissecting out the internal ear, after a subarachnoid injection, it can be easily seen that the injection mass has invaded the sac normally containing perilymph. The canals, the saccule, the utricle, and the cochlea are all outlined by the precipitation. This connection between subarachnoid space and internal ear has been generally accepted for years, and is here mentioned merely as one of the usual findings after a subarachnoid injection of either ferrocyanide solution or carbon suspension.

(*e.*) Injection of the lining membranes of the air cells. — The lining membranes of all the bony sinuses of the skull customarily show the Prussian blue precipitate after a routine cerebro-spinal injection. The coloration in these cases is apparently due to the granular deposits in the connective tissue reticulum beneath the membrane. From this supporting tissue, the granules can be made out passing into large lymph channels which drain the sinuses. Just how the fluid reaches the sinus membrane is not clear on account of the technical difficulties attending the solution of the problem. It appears more than probable that a similar process to that in the nasal cavity must hold here too, with the fluid leaving the cranial cavity along some of the numerous nerves which invade the membranes of the bony sinuses. The frontal, ethmoidal, and sphenoidal sinuses are all well injected in the routine preparation.

(*f.*) The pathway through the cervical lymph system. — To return again to the question of the fluid passage from cerebro-spinal spaces to cervical lymph channels it may be of advantage to discuss in some detail the findings in a typical routine preparation in which ferrocyanide solution has been injected for several hours under pressures slightly above the normal. After fixation of the head and neck

structures in situ and subsequent dissection, a deeply stained cervical lymph node lying high in the neck beneath the angle of the jaw becomes very prominent. The surrounding structures are not at all affected by the injection except the cranial nerves and lymph vessels. Coming into this node from above, distinct lymphatic vessels may be seen, clearly differentiated by reason of the blue precipitate within them. These apparently drain the whole base of the skull and unite into larger vessels which enter the cephalic pole of the lymph node at the mandibular angle. This entrance of the vessels into the superior pole results in this end of the node being more deeply colored than the inferior. From the node caudally, lymphatic vessels, made out by their content of the blue precipitate, can be traced in the carotid sheath.

On microscopic examination of the lymph node, the lymph vessels filled with the blue precipitate can be seen entering the gland in the vasa afferentia which invade the cortex. Just beneath the fibrous capsule the granular precipitate is massed in the small channels along the periphery. From this the granules may be traced into the substance of the node. The course of the granules is in the lymph sinuses between the trabecular strands, avoiding the various centers of germination (Fig. 11). In some exceptional instances granules of Prussian blue have been found in the lymph cells themselves; ordinarily, they are found lying free in the sinus or adhering to the outer surface of an endothelial cell.

A surprising feature of one of these lymph nodes consisted of the distribution of the granules around the arteries. Just outside of the adventitia of each of the arteries in the loose meshes of the node were masses of the Prussian blue precipitate. In the case of the smaller arteries the granules approached the lumen much more closely, but no granules were made out passing into the vascular channels. This peri-arterial distribution of the granular precipitate is perhaps the most striking feature of this one lymph node.

Another character of these lymph glands which attracts attention is the intense edema or, at least, distension of the

gland substance which occurs. This may be explained by the fact that the precipitate which results after the addition of acid represents only the one per cent salt content of the original solution, while the water of the solution plays no part in the final microscopic picture. This accounts well for the distension of the tissue spaces.

Only in one case have we found the Prussian blue precipitate in the submaxillary lymph nodes of the lower animals. Key and Retzius report this finding as constant for man, but in our preparations no constancy in this observation resulted. Probably there is some difference between man and the animals used in the general lymphatic drainage of the head sufficient to account for this disparity in the findings.

The distribution of the injection mass throughout this accessory system of drainage has been described in general for those preparations which were made by the use of ferrocyanide solutions. When suspensions of carbon granules are used with low pressure, no granules are found in the cervical lymph nodes. When, however, the injection of india ink is made at high pressure (100 mm. Hg.) the cervical nodes show in their substance carbon granules. This is probably due to the breaking down of the delicate filters which ordinarily hold back the granule substances. The microscopic preparations in these cases show the carbon granules entering along the pathway of the vasa afferentia and streaming along the lymph sinuses. The whole picture suggests that the node maintains its character as a filter for the granules, keeping them chiefly on the periphery and only in part allowing them to pass along the channels in the node itself.

(g.) The lymphatics (?) of the meninges. — This accessory or lymphatic pathway for the absorption of cerebrospinal fluid may be assumed to have its origin outside of the dura. This is substantiated by Sabin,⁴⁵ who, in the course of many injections of the lymphatics of the head, never observed any evidence of lymph vessels in the dura or within its enclosed tissues. Our preparations strongly indicate an

escape of the fluid outward along the cranial nerves, especially in the perineural spaces. From these it becomes taken up by the lymph channels after a more or less short passage through the surrounding connective tissue. Whether direct absorption by lymph vessels from the perineural space occurs is not clear, but all of our observations argue against this view.

At the time when the cerebro-spinal spaces were looked upon as similar in every respect to a serous cavity, the meninges were considered to have definite lymphatics. Arnold,² for instance, described three definite layers of lymph vessels in the pia-arachnoid. His²² accepted this view, as he was able to inject similar tissue channels. In the dura Böhm⁴ was able by puncture-injection with soluble Berlin blue to demonstrate a spread of the mass into definite channels resembling the lymphatic network in other parts of the body. But with the development of our present conception of the lymphatic system the absence of meningeal lymphatics has been proved. Sabin's⁴⁶ work is wholly against the view of lymphatic absorption in the dura itself.

There are two chief reasons for the existence of the idea that the meninges and especially the dura possesses lymph vessels. In the leptomeninges the numerous mesothelial cell-cords and the ease of injecting them, as evidenced by His' work, contributed largely to the erroneous conception. In the pachymeninx the problem is somewhat different. By puncture-injections a definite network resembling a lymphatic plexus may be easily demonstrated. This we have observed in several cases on fresh human dura, using diluted and filtered india ink as the injection mass. But this injection does not concern lymph vessels; the distended channels are merely unlined spaces between the dense strands of dural fibrous tissue. The network is wholly similar to that which may be injected in any dense fibrous layer such as *facia*.

In the dura there is another factor which contributes to the possible confusion. This is the existence of definite columns of cells, differentiated clearly in their morphology and

tinctorial reactions from the dural fibrous tissue. The evidence which has already been presented in a foregoing section leads to the view that these cell-channels existing between the fibrous strands of the dura are wholly arachnoidal in origin, and, hence, are possible pathways of circulation for the cerebro-spinal fluid. They are, however, primary pathways in relation to the veins and are not secondary or lymphatic.

(*h.*) Drainage of the spinal subarachnoid space. — The problems of fluid escape from the subarachnoid space about the spinal cord are somewhat different from those in the cranial cavity. This dissimilarity results from the absence of dural sinuses with the invading arachnoid villi in the spinal canal. Hence other plans of cerebro-spinal drainage must endure in the spinal region.

In our large series of spinal subarachnoid injections with both ferrocyanide solutions and with granular suspensions, we have never observed microscopically any evidence of escape of fluid into any of the spinal vessels — capillaries or veins. This finding is further supported by physiological observations, the data of which have been given in a foregoing subdivision of this communication. But after these typical low pressure injections of a true solution of the ferrocyanide, there is an obvious perineural deposit of precipitated granules which can be followed a short distance outward along the anterior and posterior roots. This finding accords with the reported observations of Sicard and Cestan.⁴⁸ From the perineural space about the spinal nerve roots absorption takes place along lymphatic channels. Such a mechanism of lymphatic drainage may be demonstrated in the long continued low pressure injections with the ferrocyanide method and in the replacement of one cubic centimeter of the spinal fluid with an equal amount of the ferrocyanide in an isolated portion of the spinal cord. It appears then likely that the sole pathway of fluid escape from the spinal meninges is along lymphatic channels and not directly into the blood stream. This probably eliminates constantly the cerebro-spinal fluid from the

spinal cord in the same manner as do the cephalic lymph channels.

The suggestive work of Kramer²⁷ in regard to an ascending current in the central canal of the spinal cord is of interest in any consideration of the drainage of the spinal subarachnoid space. Kramer found that if methylene blue be injected into this spinal space the tissues about the central canal were stained and the dyestuff had apparently ascended in the canal from a caudal patent metapore. The "replacement" observations which we have made with ferrocyanide are analogous in every way to Kramer's experiments and represent in some ways a better method of approach as there is no diffuse staining of the tissues with the ferrocyanide. In only one of our replacement experiments has there been evidence of this open pathway in the central canal; in the others of this series no precipitated Prussian blue granules could be found in the central canal of the spinal cord. The possibility of a connection of the central canal with the subarachnoid space by means of the perivascular channels must be excluded before any interpretation of such results can be made.

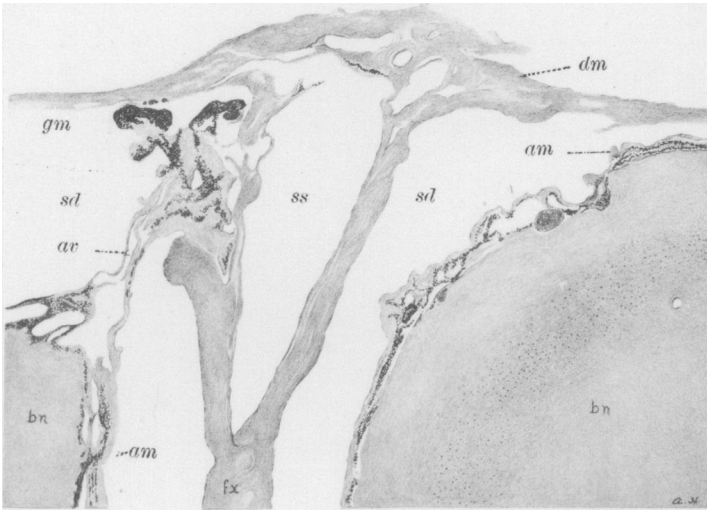
III. CONCLUSIONS.

(1.) The chief method of return of cerebro-spinal fluid to the general circulation is by a process of filtration through arachnoid villi into the great sinuses.

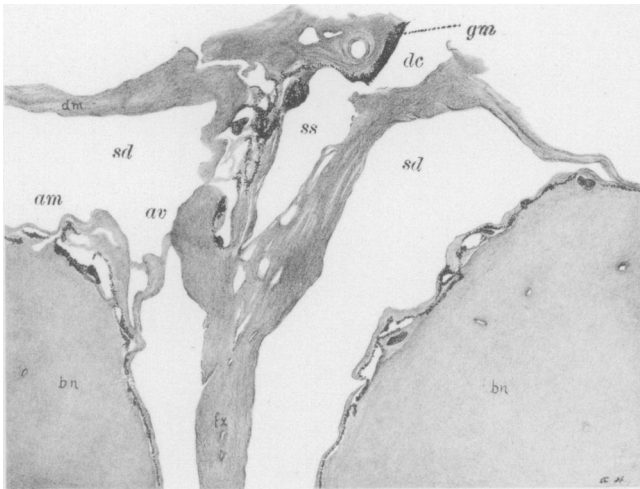
(2.) In addition to the major return of cerebro-spinal fluid by the arachnoid villi there is also an accessory drainage of the fluid into the lymphatic system. This plays a comparatively insignificant part in absorption except from the isolated spinal subarachnoid space.

(3.) No evidence has been afforded in our observations of the escape of cerebro-spinal fluid into the cerebral veins or capillaries.

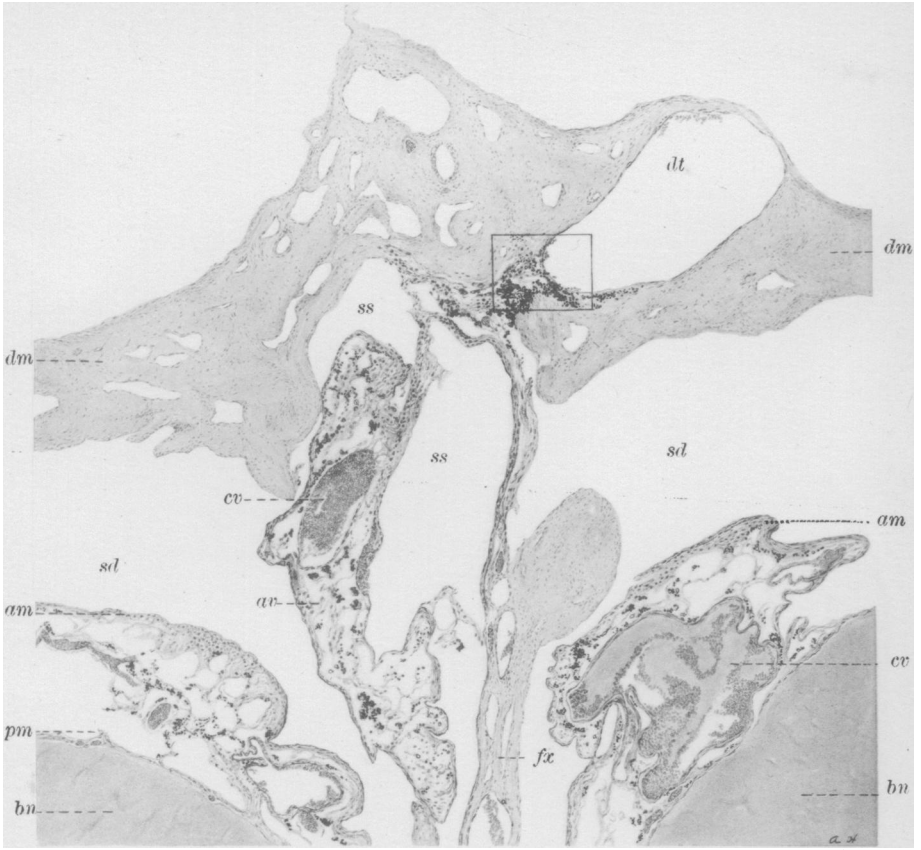
(4.) Absorption from the cranial subarachnoid space is much more rapid and much greater in amount than from the spinal portion.



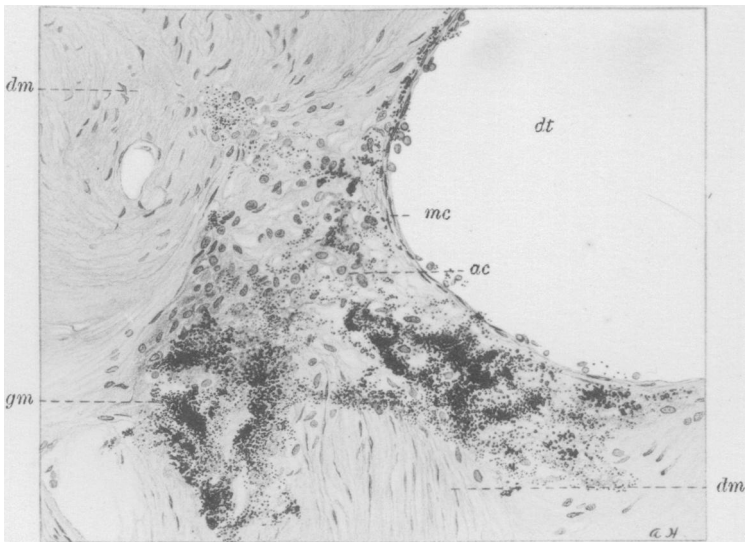
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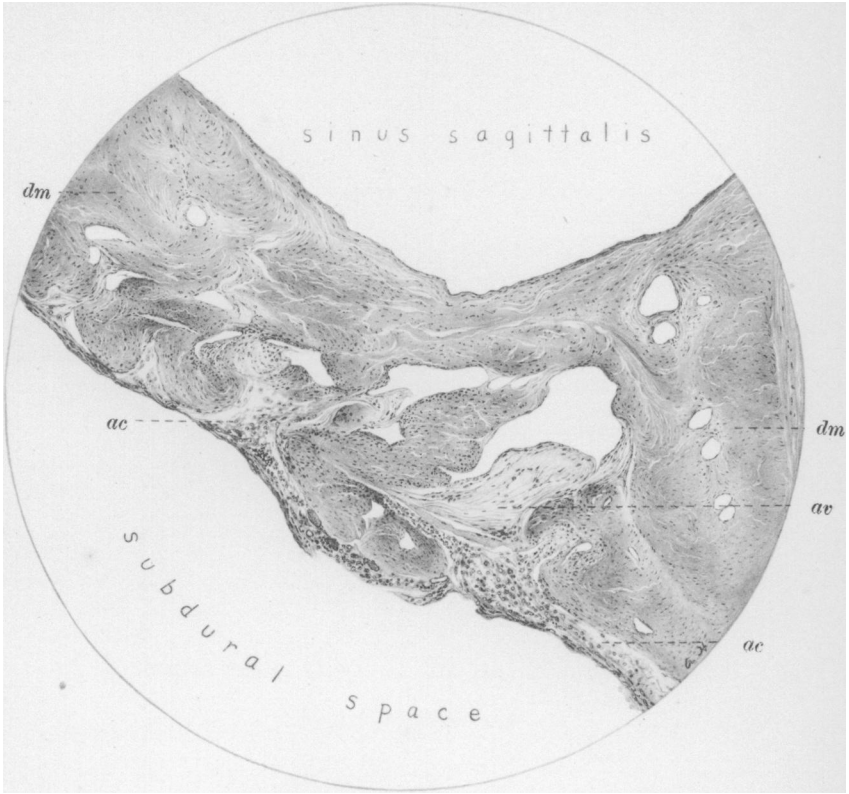
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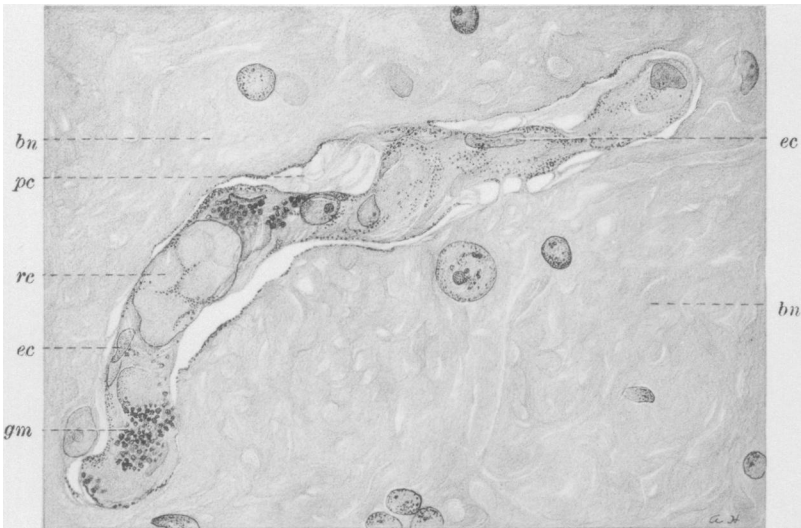
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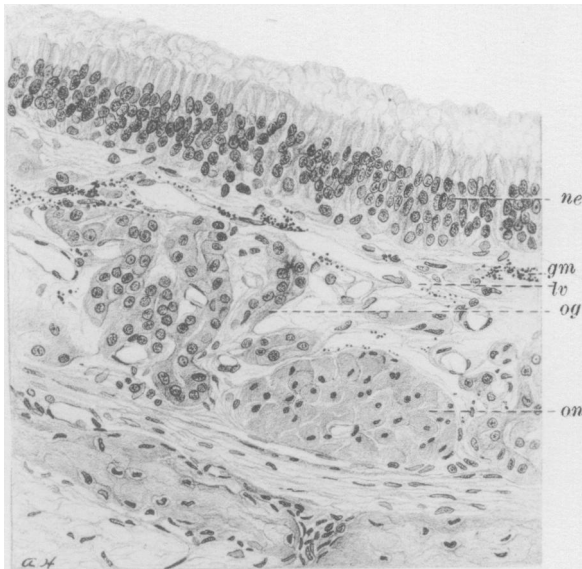
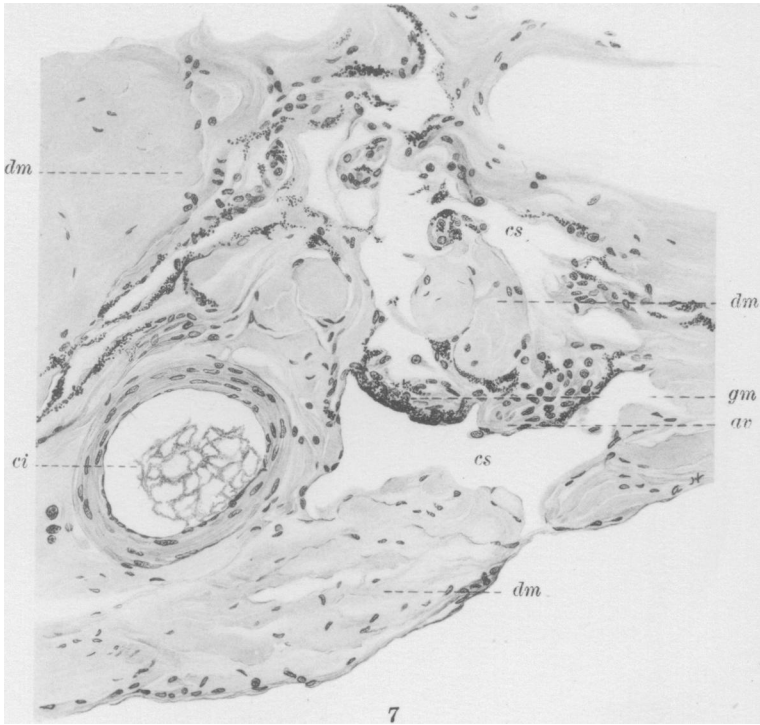
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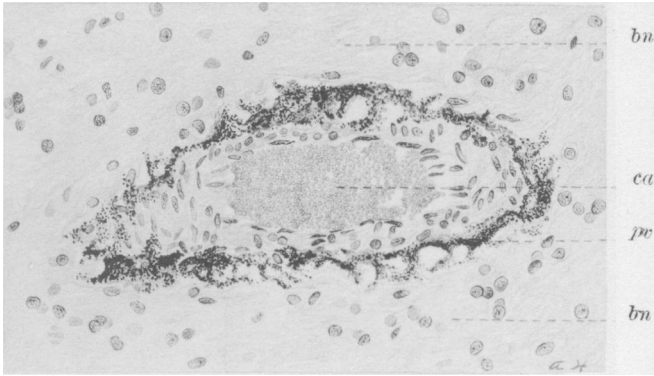


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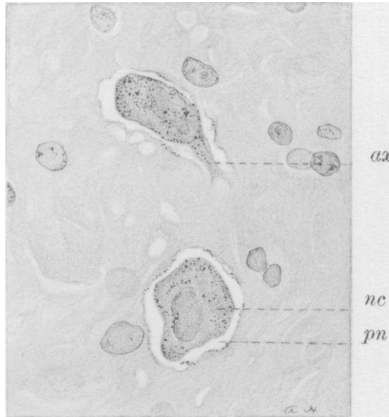


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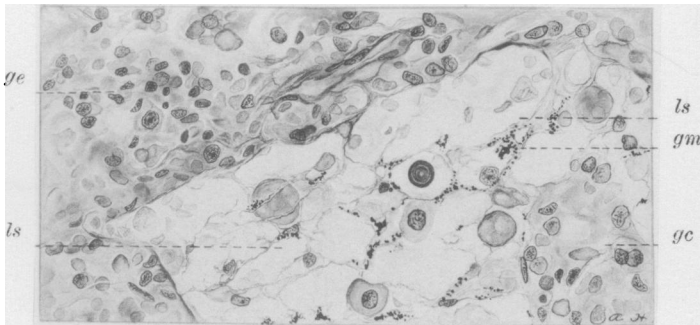




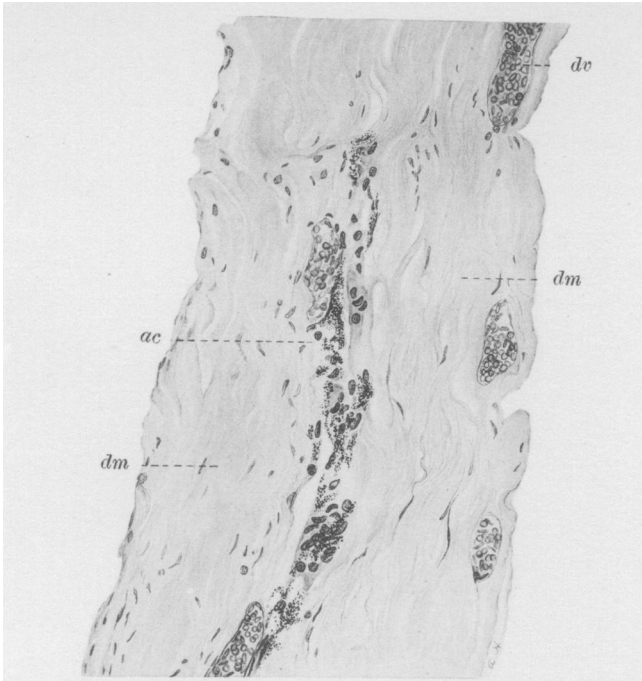
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